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Perenniporia mopanshanensis sp. nov. from China

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Abstract—A new poroid, white-rot, wood-inhabiting fungal species, *Perenniporia mopanshanensis*, is proposed based on morphological and molecular characters. This species from Yunnan Province, China, is characterized by resupinate basidiomes with a cream to buff to straw pore surface, dimitic hyphal system with strongly dextrinoid, unbranched, interwoven skeletal hyphae, and ellipsoid, non-truncate basidiospores (5.5–6.5 × 4–5 µm) with hyaline, distinctly thick, smooth, strongly dextrinoid walls. Phylogenetic analyses of ITS+nLSU sequences showed that *P. mopanshanensis* is a distinct taxon in the *Perenniporia* sensu stricto clade and is sister to *P. bannaensis*.

Key words—molecular phylogenetics, *Polyporaceae*, taxonomy, *Truncospora*, wood-rotting fungi

Introduction

Perenniporia Murrill (*Polyporaceae*, *Polyporales*) is a large cosmopolitan genus characterized by poroid basidiomata with thick-walled, ellipsoid to distinctly truncate basidiospores that are cyanophilous and with variable dextrinoid reactions (Ryvarden 1991). The *Perenniporia* hyphal system is di- or trimitic: the generative hyphae with clamp connections and the skeletal hyphae with walls that are cyanophilous and variably dextrinoid (Decock & Stalpers 2006). About 100 species are accepted in the genus (Gilbertson & Ryvarden 1987; Hattori & Lee 1999; Núñez & Ryvarden 2001; Dai & al. 2002, 2011; Cui & al. 2007; Xiong & al. 2008; Choeyklin & al. 2009; Decock 2011, 2016; Zhao & al. 2013; Ryvarden & Melo 2014; Jang & al. 2015; Ji & al. 2017).

Recently, phylogenetic studies of *Perenniporia* based on sequences of the internal transcribed spacer (ITS) region and the large subunit nuclear ribosomal RNA gene (nLSU) have revealed several well-supported clades that could be recognized as distinct genera (Robledo & al. 2009, Zhao & al. 2013). Robledo & al. (2009) demonstrated that *Perenniporia* was phylogenetically related to *Abundisporus* Ryvarden, *Hornodermoporus* Teixeira, *Perenniporiella* Decock & Ryvarden, and *Truncospora* Pilát, while Zhao & al. (2013) have shown that *Perenniporia* itself is polyphyletic. Several new species of *Perenniporia* have been described based on ribosomal DNA sequences (Zhao & Cui 2013, Jang & al. 2015, Ji & al. 2017)

During research on polypore diversity in southern China, an undescribed species of *Perenniporia* was found. We present morphological and molecular phylogenetic evidence that support the recognition of *P. mopanshanensis* as a new species.

Materials & methods

The specimens studied are deposited at the herbarium of Southwest Forestry University, Kunming, China (SWFC). Basidiomatal descriptions are based on field notes. Special colour terms follow Petersen (1996). Anatomical observations were obtained from the dried specimens and made using a light microscope following Dai (2010). The following abbreviations were used: KOH = 5% potassium hydroxide, CB = cotton blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = both inamyloid and non-dextrinoid, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n (a/b) = number of spores (a) measured from given number (b) of specimens.

The Omega EZNA HP Fungal DNA Kit was used to obtain genomic DNA from dried specimens according to the manufacturer's instructions with some modifications. A 30 mg sample from a dried fungal specimen was ground to powder with liquid nitrogen. The powder was transferred to a 1.5 ml centrifuge tube, suspended in 0.4 ml of lysis buffer, and incubated in a 65°C water bath for 60 min. After that, 0.4 ml phenol-chloroform (24:1) was added to each tube, and the suspension was shaken vigorously. After centrifugation at 13,000 rpm for 5 min, 0.3 ml supernatant was transferred to a new tube and mixed with 0.45 ml binding buffer. This mixture was transferred to an adsorbing column (AC) for centrifugation at 13,000 rpm for 0.5 min. Then, 0.5 ml inhibitor removal fluid was added in AC for a centrifugation at 12,000 rpm for 0.5 min. After washing twice with 0.5 ml washing buffer, the AC was transferred to a clean centrifuge tube, and 100 ml elution buffer was added to the middle of adsorbed film to elute the genomic DNA. ITS region was amplified with primer pairs ITS5 and ITS4 (White & al. 1990). Nuclear LSU region was amplified with primer pairs LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The ITS region

was amplified by initial denaturation at 95°C for 3 min, then 35 cycles of 94°C for 40 s, 58°C for 45 s, and 72°C for 1 min with a final extension of 72°C for 10 min. The nLSU region was amplified by initial denaturation at 94°C for 1 min, then 35 cycles at 94°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, ending with a final extension of 72°C for 10 min. The PCR products were purified and directly sequenced at Kunming Tsingke Biological Technology Limited Company. All newly generated sequences were deposited at GenBank (Table 1).

Table 1. Species, specimens, and GenBank accession numbers of studied sequences

Species name	Sample no.	GenBank accession no.		Reference
		ITS	nLSU	
A. sclerosetosus	MUCL 41438	FJ411101	FJ393868	Robledo & al. 2009
A. violaceus	MUCL 38617	FJ411100	FJ393867	Robledo & al. 2009
Donkioporia expansa	MUCL 35116	FJ411104	FJ393872	Robledo & al. 2009
Hornodermoporus "latissimus"	Cui 6625	HQ876604	JF706340	Zhao & al. 2013
H. martius	Cui 7992	HQ876603	HQ654114	Zhao & al. 2013
	MUCL 41677	FJ411092	FJ393859	Robledo & al. 2009
	MUCL 41678	FJ411093	FJ393860	Robledo & al. 2009
Microporellus violaceocinerascens	MUCL 45229	FJ411106	FJ393874	Robledo & al. 2009
Perenniporia africana	Cui 8674	KF018119	KF018128	Present study
	Cui 8676	KF018120	KF018129	Present study
P. aridula	Dai 12398	JQ001855	JQ001847	Zhao & al. 2013
	Dai 12396	JQ001854	JQ001846	Zhao & al. 2013
P. bannaensis	Cui 8560	JQ291727	JQ291729	Zhao & al. 2013
	Cui 8562	JQ291728	JQ291730	Zhao & al. 2013
P. cinereofusca	Dai 9289	KF568893	KF568895	Zhao & al. 2013
	Cui 5280	KF568892	KF568894	Zhao & al. 2013
P. corticola	Cui 1248	HQ848472	HQ848482	Zhao & al. 2013
	Dai 7330	HQ654094	HQ654108	Cui & Zhao 2013
	Cui 2655	HQ654093	HQ848483	Cui & Zhao 2013
P. ellipsospora	Cui 10276	KF018124	KF018132	Present study
	Cui 10284	KF018125	KF018133	Present study
P. hainaniana	Cui 6364	JQ861743	JQ861759	Zhao & Cui 2013
	Cui 6365	JQ861744	JQ861760	Zhao & Cui 2013
	Cui 6366	JQ861745	JQ861761	Zhao & Cui 2013
P. koreana	KUC 200932	KJ156313	KJ156305	Jang & al. 2015
	KUC 2008J-02	KJ156310	KJ156302	Jang & al. 2015
P. lacerata	Cui 7220	JX141448	JX141458	Zhao & Cui 2013
	Dai 11268	JX141449	JX141459	Zhao & Cui 2013
P. luteola	H 1308a	JX141456	JX141466	Zhao & Cui 2013
	H 1308b	JX141457	JX141467	Zhao & Cui 2013
P. maackiae	Cui 8929	HQ654102	JF706338	Zhao & Cui 2013
	Cui 5605	JN048760	JN048780	Zhao & Cui 2013
P. macropora	Zhou 280	JQ861748	JQ861764	Zhao & Cui 2013
P. medulla-panis	MUCL 49581	FJ411088	FJ393876	Robledo & al. 2009
	MUCL 43250	FJ411087	FJ393875	Robledo & al. 2009
	Cui 3274	JN112792	JN112793	Zhao & al. 2013

P. mopanshanensis	**CLZhao 5145**	**MH784912**	**MH784916**	**Present study**
	CLZhao 5152	**MH784913**	**MH784917**	**Present study**
	CLZhao 2404 [T]	**MH784911**	**MH784915**	**Present study**
	CLZhao 2311	**MH784910**	**MH784914**	**Present study**
P. nanlingensis	Cui 7620	HQ848477	HQ848486	Zhao & Cui 2013
	Cui 7589	HQ848478	HQ848487	Zhao & Cui 2013
P. pyricola	Cui 9149	JN048762	JN048782	Zhao & Cui 2013
	Dai 10265	JN048761	JN048781	Zhao & Cui 2013
P. rhizomorpha	Dai 7248	JF706330	JF706348	Zhao & Cui 2012
	Cui 7507	HQ654107	HQ654117	Zhao & Cui 2013
P. russeimarginata	Yuan 1244	JQ861750	JQ861766	Zhao & Cui 2013
P. straminea	Cui 8718	HQ876600	JF706335	Zhao & Cui 2013
	Cui 8858	HQ654104	JF706334	Zhao & Cui 2013
P. subacida	Dai 8224	HQ876605	JF713024	Zhao & al. 2013
	Cui 3643	FJ613655	AY336753	Zhao & al. 2013
	MUCL 31402	FJ411103	AY333796	Robledo & al. 2009
P. subadusta	Cui 8459	HQ876606	HQ654113	Zhao & al. 2013
P. substraminea	Cui 10177	JQ001852	JQ001844	Zhao & al. 2013
	Cui 10191	JQ001853	JQ001845	Zhao & al. 2013
P. subtephropora	Dai 10962	JQ861752	JQ861768	Zhao & Cui 2013
	Dai 10964	JQ861753	JQ861769	Zhao & Cui 2013
P. tenuis	Wei 2783	JQ001858	JQ001848	Zhao & al. 2013
	Wei 2969	JQ001859	JQ001849	Zhao & al. 2013
P. tephropora	Cui 9029	HQ876601	JF706339	Zhao & Cui 2013
	Cui 6331	HQ848473	HQ848484	Zhao & Cui 2013
P. tibetica	Cui 9459	JF706327	JF706333	Zhao & Cui 2013
P. truncatospora	Cui 6987	JN048778	HQ654112	Zhao & Cui 2013
	Dai 5125	HQ654098	HQ848481	Zhao & Cui 2013
Perenniporiella chaquenia	MUCL 47647	FJ411083	FJ393855	Robledo & al. 2009
	MUCL 47648	FJ411084	FJ393856	Robledo & al. 2009
Pe. micropora	MUCL 43581	FJ411086	FJ393858	Robledo & al. 2009
Pe. neofulva	MUCL 45091	FJ411080	FJ393852	Robledo & al. 2009
Pe. pendula	MUCL 46034	FJ411082	FJ393853	Robledo & al. 2009
Pyrofomes demidoffii	MUCL 41034	FJ411105	FJ393873	Robledo & al. 2009
Truncospora detrita	MUCL 42649	FJ411099	FJ393866	Robledo & al. 2009
T. macrospora	Cui 8106	JX941573	JX941596	Zhao & al. 2013
T. ochroleuca	Dai 11486	HQ654105	JF706349	Zhao & al. 2013
	MUCL 39726	FJ411098	FJ393865	Robledo & al. 2009
	MUCL 39563	FJ411097	FJ393864	Robledo & al. 2009
T. ohiensis	Cui 5714	HQ654103	HQ654116	Zhao & al. 2013
	MUCL 41036	FJ411096	FJ393863	Robledo & al. 2009
Vanderbylia "delavayi"	Dai 6891	JQ861738	KF495019	Zhao & al. 2013
V. fraxinea	DP 83	AM269789	AM269853	Robledo & al. 2009
	Cui 7154	HQ654095	HQ654110	Zhao & al. 2013
	Cui 8885	HQ876611	JF706344	Zhao & al. 2013
	Cui 8871	JF706329	JF706345	Zhao & al. 2013
V. "robiniophila"	Cui 5644	HQ876609	JF706342	Zhao & al. 2013
	Cui 7144	HQ876608	JF706341	Zhao & al. 2013
	Cui 9174	HQ876610	JF706343	Zhao & al. 2013
V. vicina	MUCL 44779	FJ411095	AF518666	Robledo & al. 2009

Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA) was used to edit the DNA sequence. Sequences were aligned in MAFFT 7 using the "G-INS-I" strategy (https://mafft.cbrc.jp/alignment/server/index.html) and manually adjusted in BioEdit (Hall 1999). The sequence alignment was deposited in TreeBase (submission ID 22900). *Donkioporia expansa* (Desm.) Kotl. & Pouzar and *Pyrofomes demidoffii* (Lév.) Kotl. & Pouzar were used as outgroup to root trees following Zhao & al. (2013) in the ITS+nLSU analyses.

ITS+nLSU sequence analyses were performed using maximum parsimony, maximum likelihood, and Bayesian inference methods. Maximum parsimony (MP) analyses followed Song & al. (2016), and tree construction was performed in PAUP* version 4.0b10 (Swofford 2002). All characters were equally weighted, with gaps treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees was set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each Maximum Parsimonious Tree (MPT) generated. Sequences were analyzed using Maximum Likelihood (ML) with RAxML-HPC2 through the Cipres Science Gateway (www.phylo.org; Miller & al. 2009). Branch support (BS) for ML analysis was determined by 1000 bootstrap replicates.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for each data set for Bayesian inference (BI), which was calculated with MrBayes_3.1.2 using a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 2 runs from random starting trees for 7 million generations (ITS+nLSU), and trees were sampled every 100 generations. The first 25% of the generations was discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated. Branches that received bootstrap support for maximum likelihood (BS), maximum parsimony (BT), and Bayesian posterior probabilities (BPP) greater than or equal to 75% (BS, BT) and 0.95 (BPP) were considered significantly supported.

Molecular phylogeny

The ITS+nLSU dataset included sequences from 86 fungal specimens representing 46 species (Table 1). The dataset had an aligned length of 2100 characters, of which 1553 characters were constant, 86 variable and parsimony-uninformative, and 461 parsimony-informative. Maximum parsimony analysis yielded four equally parsimonious trees (TL = 1925, CI = 0.375, HI = 0.525, RI = 0.745, RC = 0.275). Best model for the ITS+nLSU dataset estimated and applied in the Bayesian analysis: GTR+I+G. Bayesian analysis and ML analysis produced similar topologies to MP analysis, with an average standard deviation of split frequencies = 0.006258 (BI).

Perenniporia tenuis Wei 2783
Perenniporia tenuis Wei 2969
Perenniporia maackiae Cui 5605
Perenniporia maackiae Cui 8929
Perenniporia corticola Cui 1248
Perenniporia corticola Cui 1248
Perenniporia corticola Cui 2655
Perenniporia ariadne Dai 12398
Perenniporia ariadne Dai 12396
Perenniporia cinereofusca Dai 9289
Perenniporia cinereofusca Cui 5280
Perenniporia truncatospora Dai 5125
Perenniporia truncatospora Cui 6987
Perenniporia pyricola Cui 9149
Perenniporia pyricola Dai 10265
Perenniporia subtephropora Dai 10962
Perenniporia subtephropora Dai 10964
Perenniporia tephropora Cui 6331
Perenniporia tephropora Cui 9029
Perenniporia nanlingensis Cui 7620
Perenniporia nanlingensis Cui 7589
Perenniporia lacerata Cui 7220
Perenniporia lacerata Dai 11268
Perenniporia macropora Zhou 280
Perenniporia tibetica Cui 9459
Perenniporia russeimarginata Yuan 1244
Perenniporia straminea Cui 8858
Perenniporia straminea Cui 8718
Perenniporia medulla-panis MUCL 43250
Perenniporia medulla-panis MUCL 49581
Perenniporia medulla-panis Cui 3274
Perenniporia substraminea Cui 10177
Perenniporia substraminea Cui 10191
Perenniporia hainaniana Cui 6364
Perenniporia hainaniana Cui 6365
Perenniporia hainaniana Cui 6366
Perenniporia rhizomorpha Cui 7507
Perenniporia rhizomorpha Dai 7248
Perenniporia luteola Harkonen 1308a
Perenniporia luteola Harkonen 1308b
Perenniporia koreana KUC 20091030-32
Perenniporia koreana KUC20081002J-02
Perenniporia mopanshanensis CLZhao 5145
Perenniporia mopanshanensis CLZhao 5152
Perenniporia mopanshanensis CLZhao 2404
Perenniporia mopanshanensis CLZhao 2311
Perenniporia bannaensis Cui 8560
Perenniporia bannaensis Cui 8562
Perenniporia sensu stricto
100/100/1.00
85/75/1.00
100/100/1.00
96/93/1.00
100/100/1.00
100/100/1.00
100/100/1.00
-/65/-
100/100/1.00
100/100/1.00
100/100/1.00
96/95/1.00
100/100/1.00
100/100/1.00
84/77/0.98
100/100/1.00
100/100/1.00
97/93/1.00
100/100/1.00
100/100/1.00
100/100/1.00
100/100/1.00
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100/100/1.00
100/100/1.00
-/63/-
93/90/-

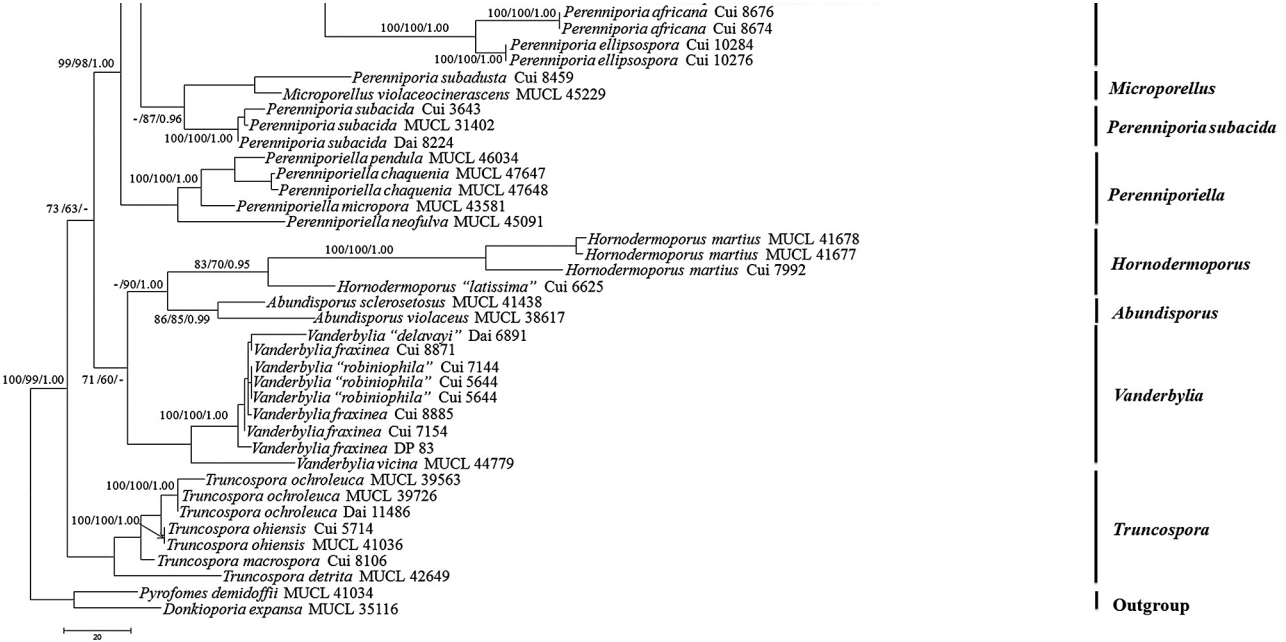

Fig. 1. Maximum Parsimony strict consensus tree illustrating the phylogeny of *Perenniporia mopanshanensis* and related species in *Polyporales* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap >70%, parsimony bootstrap proportions >50% and Bayesian posterior probabilities >0.95.

The ITS+nLSU phylogeny places *Perenniporia mopanshanensis* in the *Perenniporia* sensu stricto clade (Fig. 1), where it forms a strongly supported monophyletic lineage (BS = 100%; BT = 100%; BPP = 1) and is sister to *P. bannaensis* B.K. Cui & C.L. Zhao.

Taxonomy

Perenniporia mopanshanensis C.L. Zhao, **sp. nov**. Figs 2, 3

MycoBank MB 827580

Differs from *Perenniporia luteola* by its larger pores, its smaller basidiospores, and its unbranched skeletal hyphae.

Type: China. Yunnan Province: Yuxi, Xinping county, Mopanshan National Forestry Park, on an angiosperm trunk, 19 August 2017, C.L. Zhao 2404 (**Holotype**, SWFC 002404; GenBank MH784911, MH784915).

Etymology: The specific epithet *mopanshanensis* (Lat.) refers to the locality (Mopanshan) of the type specimen.

Basidiomata perennial, resupinate, without odor or taste when fresh, becoming corky upon drying, ≤15 × 6 cm, 8 mm thick at center. Pore surface cream when fresh, cream to buff to straw upon drying; pores round, 3–5 per mm; dissepiments thin, entire. Sterile margin narrow, cream, ≤0.5 mm wide. Subiculum cream, thin, each layer ≤0.5 mm thick. Tubes cream to buff, corky, each layer ≤2 mm long.

Hyphal structure dimitic; generative hyphae with clamp connections; skeletal hyphae strong dextrinoid, CB+; tissues unchanged in KOH.

Subiculum generative hyphae infrequent, hyaline, thin-walled, unbranched, 2–3 μm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a narrow to wide lumen, unbranched, interwoven, 2–3.5 μm in diam.

Tube generative hyphae infrequent, hyaline, thin-walled, unbranched, 1.5–3 μm in diam.; skeletal hyphae dominant, hyaline, thick-walled with a narrow to wide lumen, unbranched, interwoven, 2–3.5 μm. Plenty of crystals present among hyphae. Cystidia absent, fusoid cystidioles present, hyaline, thin-walled, 13–19 × 4.5–6.5 μm; basidia barrel-shaped to clavate, with four sterigmata and a basal clamp connection, 14–20 × 7–12 μm; basidioles dominant, mostly pear-shaped, but slightly smaller than basidia.

Basidiospores ellipsoid, non-truncate, hyaline, distinctly thick-walled, smooth, strong dextrinoid, CB+, (5–)5.5–6.5(–7) × 4–5(–5.5) μm, L = 6.15 μm, W = 4.6 μm, Q = 1.35–1.42 (n = 120/4).

Type of rot: white rot.

Additional specimens examined: **CHINA. Yunnan province. Yuxi:** Xinping county, Mopanshan National Forestry Park, on the angiosperm trunk, 19 August 2017,

Fig. 2. *Perenniporia mopanshanensis* (holotype, SWFC 002404). Basidiomata. Scale bar: 3 cm.

C.L. Zhao 2311 (SWFC 002311; GenBank MH784910, MH784914); 13 January 2018, C.L. Zhao 5145 (SWFC 005145; GenBank MH784912, MH784916); C.L. Zhao 5152 (SWFC 005152; GenBank MH784913, MH784917).

Discussion

The new species, *Perenniporia mopanshanensis,* is supported by phylogenetic analyses and morphological characters. In the ITS+nLSU analyses (Fig. 1), it forms a strongly supported monophyletic lineage (BS = 100%; BT = 100%; BPP = 1) where it forms a clade with *P. bannaensis*. However, morphologically *P. bannaensis* differs from *P. mopanshanensis* by its annual basidiocarps with buff-yellow to pinkish buff pore surface and smaller pores (6–8 per mm; Zhao & al. 2013).

Morphologically, the presence of non-truncate basidiospores is shared by several other species in *Perenniporia* sensu stricto: *P. africana* Ipulet & Ryvarden, *P. ellipsospora* Ryvarden & Gilb., *P. koreana* Y. Jang & J.J. Kim, *P. luteola* B.K. Cui & C.L. Zhao, *P. rhizomorpha* B.K. Cui & al., and *P. subacida* (Peck) Donk. A morphological comparison between *P. mopanshanensis* and these six species is presented in Table 2.

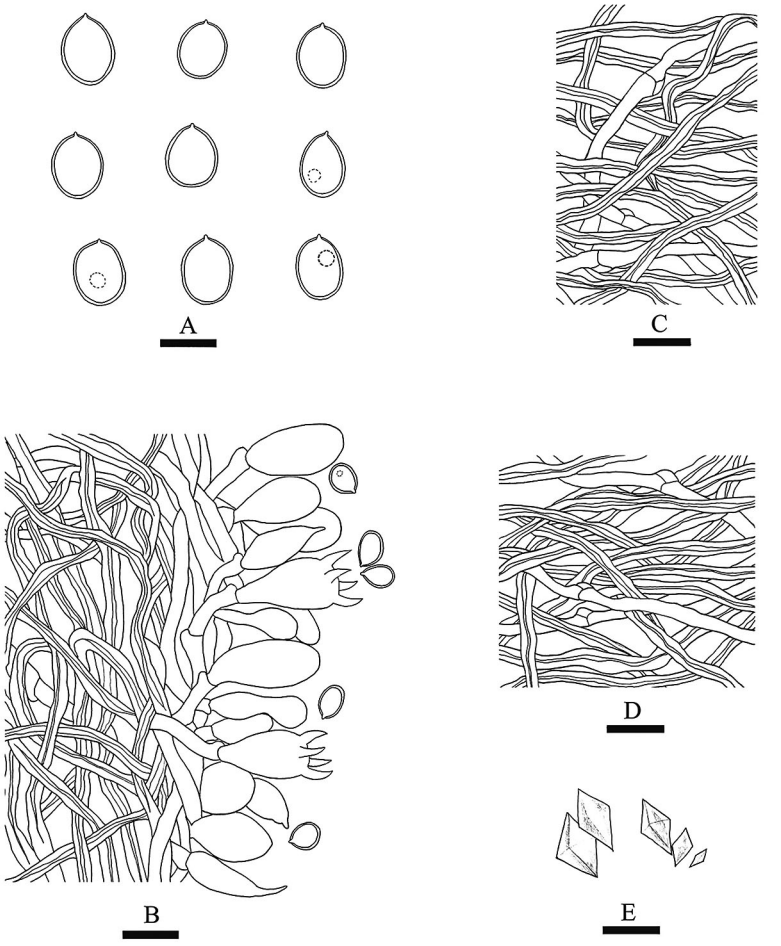

Fig. 3. *Perenniporia mopanshanensis* (holotype, SWFC 002404). A. Basidiospores; B. Section of hymenium; C. Hyphae from trama; D. Hyphae from subiculum; E. Rhombic crystal. Scale bars: a = 5 μm; b–e = 10 μm.

Polypores are an extensively studied group in *Basidiomycota* (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Ryvarden & Melo 2014), but Chinese polypore diversity is still not well known, especially in the subtropics and tropics. The new species, *Perenniporia mopanshanensis,* was found in the Chinese subtropics, where many new taxa in the *Polyporales* and *Hymenochaetales* have

Table 2. A comparison of *Perenniporia* species with non-truncate basidiospores

Species	Habit	Pore surface	Pores / mm	Spores (µm)	Skeletal hyphae	Reference
africana	Annual	Pale orange to brown	6–8	4–5 × 3–4	Branched	Ipulet & Ryvarden 2005
ellipsospora	Annual	Whitish to pale yellowish brown	3–4	4–5.5 × 3–4	Unbranched	Gilbertson & Ryvarden 1987
koreana	Annual	Grayish orange	5–6	6–7 × 3.9–5.2	Rarely branched	Jang & al. 2015
luteola	Perennial	Buff-yellow	4–6	6–7 × 5–5.5	Frequently branched	Zhao & Cui 2013
mopanshanensis	Perennial	Cream, buff to straw	3–5	5.5–6.5 × 4–5	Unbranched	This study
rhizomorpha	Annual	Yellow-buff to yellowish orange	4–6	5.3–6.5 × 4.1–5.2	Branched	Cui & al. 2007
subacida	Perennial	Ivory to yellowish	5–6	4.5–6 × 3.5–4.5	Unbranched	Decock & Stalpers 2006

been described (Cui & Dai 2008; Cui & al. 2009, 2010, 2011; Du & Cui 2009; Li & Cui 2010; He & Li 2011; Jia & Cui 2011; Yu & al. 2013; Yang & He 2014; Chen & al. 2015). We anticipate that additional polypore taxa will be found in China after further investigation and molecular analysis.

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